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13. ABSTRACT

This paper was presented at the Proceedings of the 1st Annual Conference on Environmental Toxicology, sponsored by the SysteMed Corporation and held in Fairborn Ohio on 9, 10, and 11 September 1970. Major technical areas discussed included toxicological evaluation of carbon monoxide, methodology, pathology, atmospheric contaminants, and toxicology of propellants and other military chemicals.

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PAPER NO. 5

ACUTE TOXICITY OF CARBON MONOXIDE UNDER HYPERBARIC CONDITIONS

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INTRODUCTION

The increasing importance of manned exploration under the sea has necessitated the development of data pertaining to the toxicity of contaminants in confined spaces under hyperbaric conditions. In any discussion of the effects of contaminants under pressure, carbon monoxide (CO) generates more than routine interest because of its unique mode of action and its numerous sources of generation--including production by man himself. The importance of considering atmospheric contaminants and their effects under pressure was emphasized by the identification of carbon monoxide in the breathing atmosphere of Sealab II in concentrations as high as 30 ppm at the surface.

A wealth of information has accumulated regarding the effect of CO on living systems at normal atmospheric pressure. In addition, studies have been conducted at hypobaric conditions. There is, however, little if any data pertaining to the acute toxicity of CO in intact animals under hyperbaric conditions. Therefore, the toxicity of carbon monoxide was evaluated under conditions of elevated pressure to determine if a pressurized environment would result in an altered response of an animal to the gas.

The toxicity of oxygen at high partial pressures is well recognized. No untoward effects, however, are noted when the partial pressure of oxygen is maintained at approximately 160 mm Hg regardless of the overall pressure of the system. It was reasoned, therefore, that the toxicity of carbon monoxide should depend solely on the number of molecules presented to the alveoli, if the partial pressure of oxygen in the environment remained constant.

Two criteria were utilized to evaluate CO toxicity: (1) a comparison of the LC_{50} of carbon monoxide at ambient atmospheric pressure and at elevated pressures, and (2) the percent saturation of blood hemoglobin with carbon monoxide (carboxyhemoglobin) in animals which died during the exposures.

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Experimental Animals

The animals utilized in these studies consisted of male Sprague-Dawley-derived rats, male Swiss albino mice, and male Hartley-derived guinea pigs. Prior to exposure, the animals were maintained on the appropriate food and water ad libitum. No food or water was allowed during the exposure.

Exposure Equipment and Materials

Exposures at ambient atmospheric pressure were conducted in a 30 liter cylindrical plexiglass chamber. A predetermined amount of carbon monoxide was mixed with 7.1 liters per minute of laboratory air and introduced into the chamber. The system used is shown schematically in figure 1.

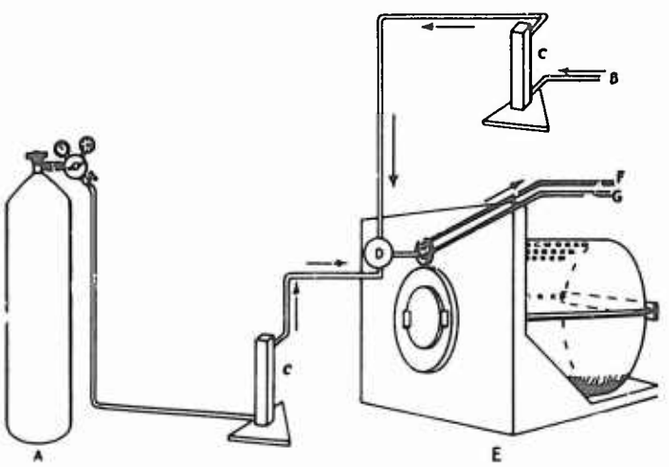

Figure 1. SCHEMA OF EXPOSURE SYSTEM USED AT 0 PSIG. A, carbon monoxide cylinder (C.P. Grade) and regulator; B, house air for dilution; C, flowmeters; D, mixing flask; E, exposure chamber; F, exhaust line; G, sampling line.

The hyperbaric studies were conducted in an 8.6 liter Bethlehem Model 614 chamber rated for a maximum pressure of 150 psig at 70 F. For these exposures, certified premixed cylinders of carbon monoxide, oxygen, and helium were procured. Additional intermediate concentrations were mixed, as required, from the primary cylinders. During all runs, the partial pressure of oxygen was maintained between 140 and 160 mm of Hg by decreasing the oxygen from 21% (0 psig) to 7.6% (25 psig), 4.6% (50 psig), 3.3% (75 psig), and 2.6% (100 psig). In a similar manner, the various

concentrations of carbon monoxide in the gas mixtures introduced into the chamber for the LC_{50} determinations had to be lowered concomitantly with the stepwise increases in pressure.

The pressure in the chamber was continuously monitored using a top mounted pressure gauge with a range of 0 to 100 psig. During exposures, chamber pressures were maintained within ±0.5 psig of the desired pressures. The gaseous mixtures containing carbon monoxide were dynamically fed into the chamber through 1/4 inch flexible tubing and an exhaust flow rate of 4 to 5 liters per minute was maintained during the exposure period. Following all exposures, the chamber was decompressed with a mixture of 79% helium and 21% oxygen at a predetermined uniform rate depending on the experimental pressure. Rapid decompression was not used since at times one or more animals would not be visible through the chamber window at the termination of an exposure and an accurate death count would not have been obtained. The system used is presented diagrammatically in figure 2. The thermal variation of the chamber atmosphere as measured by a thermocouple, did not exceed ± 2 C of room temperature (23 C) during the animal exposure and decompression phases.

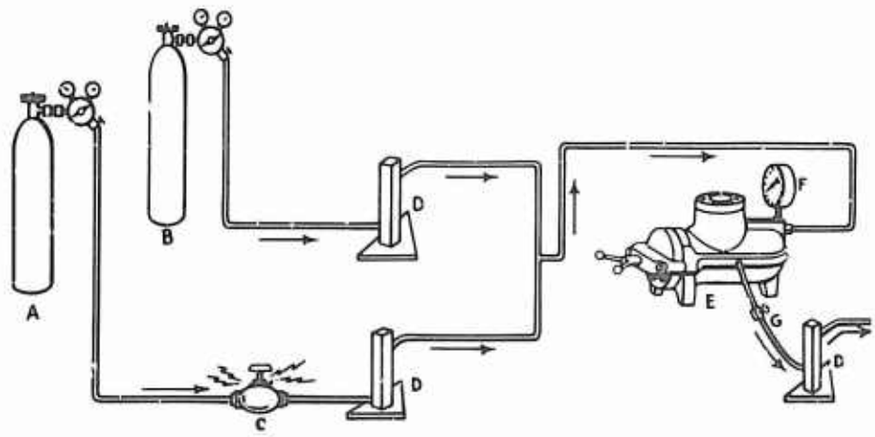

Figure 2. SCHEMA FOR HYPERBARIC EXPOSURES AT 25, 50, 75 AND 100 PSIG. A, cylinder containing decompression mixture of 79% helium, 21% oxygen with regulator; B, cylinder containing mixtures of carbon monoxide, oxygen, and helium with regulator; C, motorized valve; D, flowmeters; E, hyperbaric chamber; F, pressure gauge; G, exhaust flow regulator valve.

In both the 0 psig and hyperbaric studies, chamber loadings consisted of 4 rats, 4 guinea pigs, or 16 mice and all exposures were of 4 hour duration. A 4 hour exposure period was arbitrarily selected because of its general use in this and other laboratories in acute LC_{50} studies with other materials at normal atmospheric pressure.

Immediately following the exposures at 0 psig or the decompression phase after the hyperbaric runs, cardiac blood samples were collected from the dead rats and guinea pigs and analyzed for carboxyhemoglobin concentration. The surviving animals were not observed further and were sacrificed.

Analytical Techniques

In all exposures at normal atmospheric pressure, the actual concentration of CO was continuously monitored throughout the 4 hour period by a non-dispersive double beam infrared spectrophotometer set at a wavelength of 2160 cm^{-1} and using a 5.65 liter variable pathlength gas cell. All laboratory mixed cylinders for the hyperbaric exposures were analyzed for carbon monoxide concentrations by the infrared spectrophotometer or by a gas chromatograph using a nickel oxide catalyst which reduced CO to methane. No monitoring during the run was considered necessary.

Carboxyhemoglobin Method

Blood carboxyhemoglobin concentration was determined by a method based on the work of Stowe and Pelletier (1968) using a two-channel automatic chemical analyzer. A specimen of blood, with ethylenediaminetetraacetic acid as the anticoagulant, was split into two streams. Total hemoglobin was determined in one stream as cyanmethemoglobin at 550 mμ, and the carbon monoxide was released from hemoglobin in the other stream with 10% H_2SO_4. The gas phase was then removed with a trap and reacted with the silver salt of p-sulfaminobenzoic acid in alkaline solution. This resulted in a colloidal solution of silver which was measured spectrophotometrically at 420 mμ.

RESULTS AND DISCUSSION

In general, all animals lost consciousness during the first 1 to 2 hours of carbon monoxide exposure. Table I presents the LC_{50} values and their 95% confidence limits at pressures from 0 to 100 psig. As can be seen, there is very little variation in the absolute amount of carbon monoxide at the 50% mortality level in rats, guinea pigs and mice as the pressure is increased in stepwise fashion from 0 to 100 psig. Figure 3 is a pictorial representation of the data presented in table I. The small variation in the CO LC_{50} values between pressure levels within a particular species was not considered to be biologically significant. It was also noted that guinea pigs were considerably less susceptible to CO intoxication at all pressures than were the rats and mice.

TABLE I

LC50 Values for Carbon Monoxide
at Pressures from 0-100 psig

PSIG	RATS CO LC50 (mg/m³)	GUINEA PIGS CO LC50 (mg/m³)	MICE CO LC50 (mg/m³)
0	2070 (1831-2341)[a]	6550 (5509-7788)	2800 (2679-2926)
25	2670 (2278-3129)	6800 (5888-7399)	2700 (2457-2967)
50	2500 (2372-2635)	8300 (7339-9387)	2230 (2046-2431)
75	2680 (2354-3060)	7000 (5902-8302)	2800 (2528-3101)
100	2500 (2385-2620)	7900 (6884-9132)	2270 (2202-2340)

[a] 95% confidence limits.

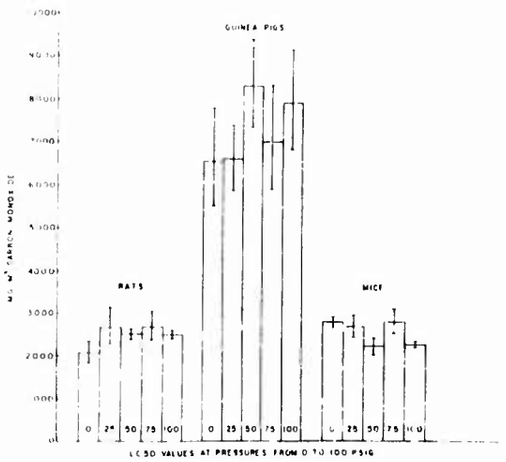

Figure 3. LC_{50} OF CARBON MONOXIDE IN RATS, GUINEA PIGS, AND MICE AT 0, 25, 50, 75 AND 100 PSIG.

This phenomenon correlates with the lower affinity constant of hemoglobin for CO in guinea pigs than in either rats or mice. The approximate ratios of the partial pressures of CO to O_2 at the LC_{50} are given in table II as well as the mean carboxyhemoglobin values in rats and guinea pigs which died during the exposures at each pressure. Both the COHb levels producing death in rats and guinea pigs and the partial pressure gas ratios exhibited little variation regardless of the total pressure of the exposure environment.

TABLE 2

Partial Pressure Gas Ratios and Carboxyhemoglobin Percentages[a] for Rats and Guinea Pigs Exposed to Carbon Monoxide at Pressure

	RATS		GUINEA PIGS	
psia	pCO/pO2 for LC50	% COHb Mean±S.D.	pCO/pO2 for LC50	% COHb Mean±S.D.
0	0.009	57.5±6.9	0.029	77.6±10.4
25	0.012	62.1±15.8	0.029	71.5±19.8
50	0.011	60.7±5.7	0.036	67.5±16.1
75	0.012	68.8±8.7	0.031	67.6±12.9
100	0.012	64.9±9.8	0.036	65.0±8.3

[a] Values based on all concentrations of CO which resulted in animal deaths at a particular pressure

Berger et al (1964), using a carbon monoxide-air mixture, has shown in vitro that the equilibrium percentage of blood carboxyhemoglobin produced by a given concentration of CO was independent of the environmental pressure. More recently it has been demonstrated that the relative affinity constant of hemoglobin for carbon monoxide from both whole blood and prepared hemoglobin solutions was not significantly affected by elevated pressure or the inert gas component of the pressurized atmosphere (Rodkey et al, 1969). The carboxyhemoglobin values obtained from intact animals at death in the present studies support these findings.

Henderson and Haggard (1943) indicated that at equilibrium the distribution of hemoglobin between carbon monoxide and oxygen depended on the ratio of the partial pressures of CO to O_2 as well as the affinity of hemoglobin for these two components. Berger et al also suggested that the apparent toxicity of CO should be unaffected if the ratios of these two gases remained constant. The partial pressure gas ratios reported in table II support this hypothesis.

It is anticipated that many additional atmospheric contaminants will be encountered in manned exploration under the sea. The results reported here indicate that the toxicity of carbon monoxide is not altered by increases in ambient pressure up to approximately 8 ATA provided the partial pressure of oxygen in the atmosphere remains constant. Carbon monoxide, however, is unique in its mode of action and no attempts were made to evaluate any subjective effects, chronic effects, behavioral effects or measurements of decrements in performance during the exposures. Therefore, one should not generalize from data on carbon monoxide as to the toxicity of other materials under hyperbaric conditions.

This presentation is based on a paper published in the Journal of Toxicology and Applied Pharmacology, Vol. 17, p. 752-760, November 1970.

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DISCUSSION

DR. MAC FARLAND (York University): The LC_{50} values you gave are four hour LC_{50}'s?

LIEUTENANT ROSE (National Naval Medical Center): Right. Four hour exposures.

DR. MAC FARLAND: Did you ever observe a death after the exposure was terminated?

LIEUTENANT ROSE: All surviving animals were sacrificed immediately following exposure.

DR. MAC FARLAND: That's not my question. Did you ever observe a death after the exposure was terminated?

LIEUTENANT ROSE: During the decompression one time, the decompression period after the CO was shut down, there was one death in the guinea pigs. That was not tabulated in the LC_{50} data, but that was the only time.

DR. MAC FARLAND: Yes, you see the question in my mind is this, carbon monoxide is very unusual, it is easy to determine the LT_{50} for carbon monoxide exposures, but the LC_{50} is difficult because as soon as you terminate the exposure, essentially the animals now start to recover. From the curves we saw in the preceding paper, as soon as the exposure is terminated the animal begins to blow off carbon monoxide so that deaths postexposure are almost unknown and this seems to have been your experience. I have a feeling that the proper determination of the LC_{50} with carbon monoxide for a stated exposure period, say four hours, would involve conducting exposures, the duration of which was longer than four hours in order to get the top points on the regression line above the LC_{50}, but I'm not certain of this, but carbon monoxide is unique in this regard, it is an interesting problem. Thank you.

MR. HAUN (SysteMed Corporation): May I ask what were the numbers of animals in each group and the weights of the animals?

LIEUTENANT ROSE: The weights of the rats ranged between 225 and 300 grams; the guinea pigs between 300 and 350, and mice between 25 and 28 grams. I don't remember the exact figures for total number of animals utilized. For rats, it was approximately 120, guinea pigs approximately 80. For mice it was considerably more because we used 16 for exposures instead of four.

MR. HAUN: The numbers per group exposed were how many?

LIEUTENANT ROSE: Approximately 30 to 40 per group at any one pressure.